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The crosstalk between microbial sensors ELMO1 and NOD2 shape intestinal immune responses

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Microbial sensors are integral to maintaining cellular homeostasis and orchestrating immune responses within the intestinal environment. The mechanisms contributing to differential immune responses and their link to inflammatory bowel diseases (IBD) are not yet fully understood. IBD is an umbrella term used to describe disorders that cause chronic inflammation of the gastrointestinal tract. This review explores The crosstalk between microbial sensors ELMO1 and NOD2 shape intestinal immune responses during enteric infections of AIEC-LF82 and Salmonella published in the February 2023 Virulence Journal by Aditi Sharma *et al.*

The two most common forms are ulcerative colitis and Crohn's disease (CD).[1] Recently, ELMO1 (Engulfment and Cell Motility Protein-1) has emerged as a critical player in this process, working in direct association with the bacterial sensor protein, Nucleotide-binding oligomerization domain-containing protein 2 (NOD2). ELMO1 has been implicated in the inflammatory cascade of IBD by sensing microbes associated with NOD2 and triggering pro-inflammatory cytokines secretion. Mutations in NOD2, an intracellular receptor for the bacterial cell wall component muramyl dipeptide (MDP),[2] are among the strongest risk factors for disease.[3] The most common mutation, a frameshift of NOD2 (L1007fs) resulting in premature termination, was previously found to be defective in its recognition of MDP.[4] Until now the direct interaction between ELMO1 and NOD2 regulating bacterial sensing was unknown. This knowledge has the potential to modernize our approach to preventing and treating NOD2-mediated inflammatory bowel diseases, and other immune-related disorders. Effective bacterial sensing is a critical first-line defense against infection and impairments in this process have been linked to the development of several autoimmune and inflammatory conditions, including CD.[5,6] pattern recognition receptors like ELMO1 and NOD2 are essential for microbial sensing between commensals and pathogens by identifying pathogen-associated molecular patterns associated with microbes.[7] In this study Sharma *et al*., report the direct link between these two important microbial sensors, and the role they have in determining host response to pathogens. By using a stem-cell approach to stimulate normal gut physiology, and intestinal bacteria (*Salmonella*), Adherent-invasive *E. coli* strain LF82 (AIEC-LF82) to assess the guts' function to stress, due to its association with CD. They found that the C-terminal region of ELMO1 was sufficient for interaction with the Leucine-Rich Repeat (LRR) region of NOD2 and that the absence of either or both proteins results in dysregulated antibacterial response in the case of AIEC-LF82 and *Salmonella* infection.

They started by assessing the physical interaction of the C-terminal domain of ELMO1 with NOD2. The C-terminal region of ELMO1 is known to be essential for bacterial phagocytosis due to the interaction with DOCK 180 by its PH domain regulating the GTP-ase, Rac, which is involved in cytoskeletal remodeling.[8] The C-terminal of ELMO1 binds with bacterial effectors through the signature WxxxE motif to induce various immune responses between pathogens and commensals by interacting with several bacterial effectors.[9] Similarly, the LRR domain of NOD2 recognizes bacterial components and was identified in the binding of the bacterial cell wall component MDP.[10,11] The interaction between ELMO1 and the LRR region of NOD2 suggests a coordination between the two sensing systems that may have significant effects on the immune system's ability to detect and respond to bacteria by influencing bacterial recognition, bacterial engulfment and clearance, and regulation of the immune response.

They next examined the functional effects of the CD-associated L1007fsinsC NOD2 mutant in epithelial and immune cells through ELMO1-NOD2 interactions. This mutant has previously been shown to reduce NFκB activity compared to wild-type NOD2.4 In, this study Sharma *et al*. found that the Leucine frame-shift mutation did not interrupt ELMO/NOD2 binding, yet did impede NFκB activity and reduce the phagocytosis of bacteria while.

Although initial phagocytosis of bacteria was reduced in knockout cells, these cells were later found to contain more bacteria than their wt counterparts indicating that while cells could still phagocytize some bacteria, they were unable to eliminate these organisms.

To investigate why the bacteria were able to persist in immune cells, the pro-inflammatory cytokines, MCP-1, IL-6, and IL-8, secreted by these cells and Reactive Oxygen Species (ROS) levels induced within these cells were examined. Indeed, depleting ELMO1 and NOD2 resulted in reduced pro-inflammatory cytokine levels in response to enteric pathogens and decreased ROS levels. This suggests that ELMO1 and NOD2 play a role in regulating the inflammatory response and ROS production, which are important for combating bacterial infections. The depletion of these proteins impairs the ability of cells to mount effective immune responses, making them more susceptible to bacterial infection and persistence.

While this research provides new insights, it was limited to specific bacterial challenges and a small number of NOD2 and ELMO1 mutations, including complete knockouts and specific CD-associated mutations.

Sharma et al. are the first to report a direct interaction between NOD2 and ELMO1, important microbial sensors, which play a significant role in determining host response to pathogens. These direct interactions of NOD2 and ELMO1 influence the course of bacterial infection by regulating bacterial survival/clearance, ROS generation, and immune responses during AIEC-LF82 and Salmonella infections. Future research should focus on uncovering the structural and molecular details of this interaction and the subsequent pathway involved. This knowledge can potentially reveal alternative therapeutic targeting CD where defective sensing of luminal bacteria contributes to the disease's pathogenesis.

Taken together, a role for ELMO1 might be to stabilize NOD2 in an active conformation where it can interact with MDP to elicit an acute, transient upregulation of pro-inflammatory signals including IL-6 and ROS. Unlike the knockout NOD2s, mutant proteins associated with CD, such as L1007fs, may, with the assistance of ELMO1, be unable to refold into an inactive form (**Figure 1**). While trapped in an active conformation, NOD2 continues to promote pro-inflammatory signals, even in the absence of infection, resulting in chronic inflammatory conditions.

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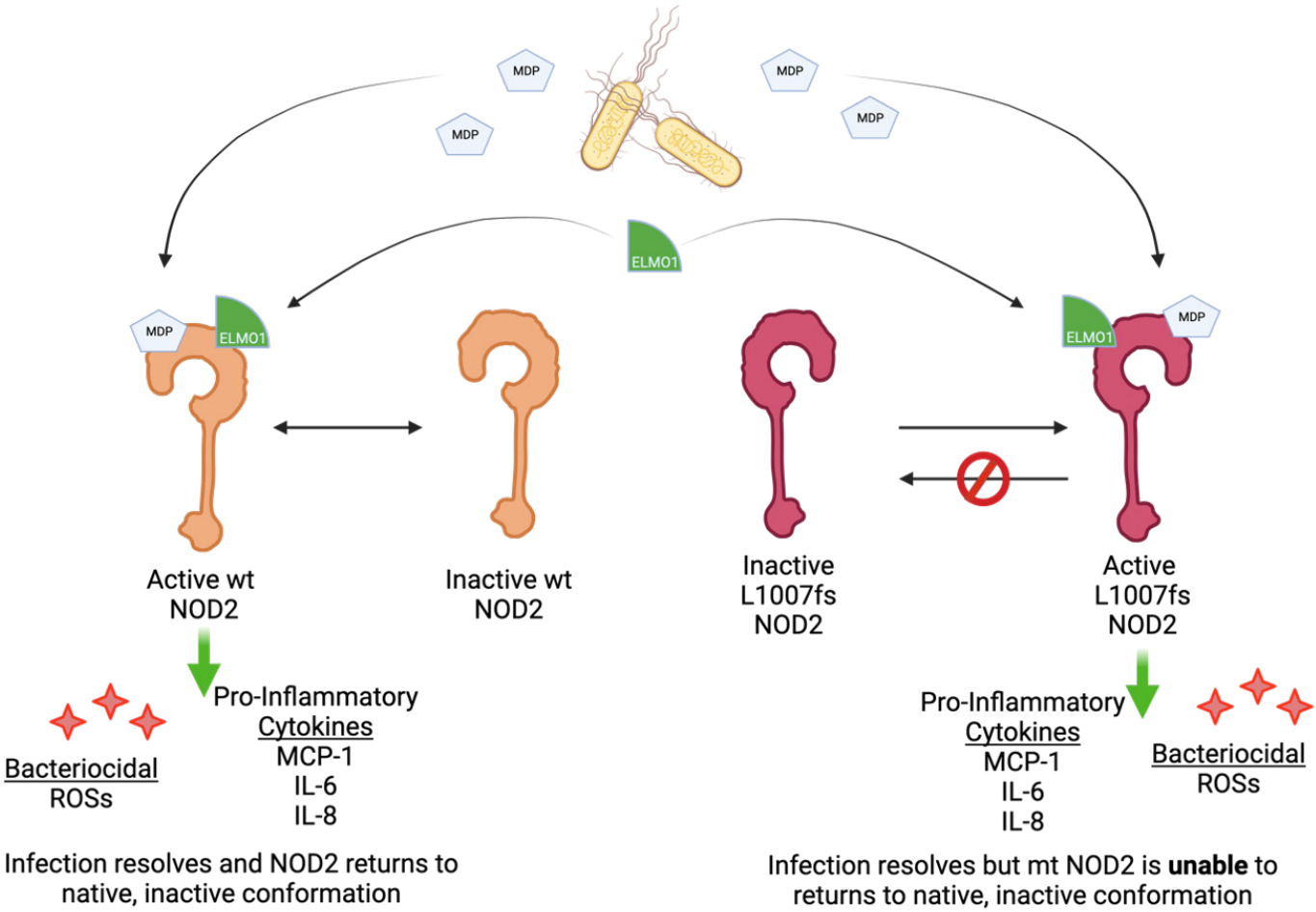

Figure 1 ELMO1 binding stabilizes NOD2 in an active conformation | ELMO1 binds wt NOD2 at the LRR domain to transiently upregulate inflammatory and bacteriocidal reactions. ELMO1 binds L1007fs NOD at the LRR domain to chronically upregulate inflammatory and bacteriocidal reactions resulting in CD and other chronic inflammatory conditions.